simulations. The mutant models were obtained by mutating the NMR minimized average structure of αS (PDB ID = 1XQ8). Atomistic simulations for both wild-type and mutated αS in explicit water were conducted for at least 30 ns at four different temperatures (i.e., 310 K, 330 K, 350 K and 372 K) using the CHARMM22/CMAP force field. For wild-type αS, the percentage of α-helix changed by less than 5% from that of the NMR structure regardless of simulation temperature. For each mutant αS, the percentage of α-helix differed by 10% or less regardless of simulation temperature. In all simulations, the acidic tail of αS remained predominantly random coil. Moreover, at 372 K, beta sheet was temporarily observed within a small region (approximately 3%) for the wild-type and A30P mutant.

2300-Pos
Protein Trajectory Reconstruction Strategies from FRET-Derived Distance Constraints
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Critical to almost all biological processes, proteins are truly the cell's functional building blocks. The remarkable specificity and diversity of protein function are consequences of native conformational fluctuations, or protein dynamics, in the aqueous cytoplasmic environment. While crystallographic and NMR-based methods have dramatically improved our structural interpretation of proteins, extending that knowledge to predict and describe these dynamic processes remains a major open challenge. Merging Förster Resonance Energy Transfer (FRET) data from single molecule experiments with Elastic-Network protein Models (ENM), we reconstruct dynamic adenlyate kinase (ADK) trajectories over extended time scales and throughout its catalytic process.
With exceptional temporal resolution, single-molecule FRET experiments can produce inter-residue distance traces between modified residues within a single protein. Here, this distance is treated as a perturbation of the equilibrium distance from the PDB structure, reduced to an elastic network with only one alpha-carbon atom at each node. Normal Mode Analysis (NMA) reveals the natural directions of motion most responsible for the (inter-residue) distance perturbation, and these same eigen modes inform the geometry of all other (non-tagged) nodes. By reconstructing the entire protein network at each FRET time point, we have determined a maximum-likelihood estimation of the protein trajectory and can analyze the spatial dynamics of regions both proximal and distal to the tagged residues. Moreover, we suggest an automated solution for the non-trivial dye placement of fluorophore pairs that maximize information content of resultant distance traces. The combination of FRET data and ENM techniques access timescales beyond those of MD, but we show agreement between our method and atomistic ADK simulations from different starting structures in smaller temporal windows.

2301-Pos
Conserved Hydrophobic and Hydrophilic Bond Interaction Networks in ErbB Family Kinases
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The EGFR/ErbB/HER family of kinases contains four homologous receptor tyrosine kinases that are important regulatory elements in key signaling pathways. To elucidate the atomistic mechanisms of activation in the ErbB family, we perform molecular dynamics simulations on the three members of the ErbB family with known kinase activity, EGFR, HER2 (ErbB2) and ErbB4, in different molecular contexts: monomer vs. dimer, wildtype vs. mutant. Using bioinformatics and fluctuation analyses of the molecular dynamics trajectories, we relate sequence similarities to similarities in specific bond-interaction networks and similarities in collective dynamical modes. We find that in the active conformation of the ErbB kinases, key loop motions are coordinated through conserved hydrophilic interactions, an activating bond-network consisting of hydrogen bonds and salt bridges. While the inactive conformations show a less extensive inactivating bond network, they sequester key residues and disrupt the activating bond-network. Both conformational states are also stabilized through context-specific hydrophobic interactions. We show that the functional (activating) asymmetric kinase dimer interface forces a corresponding change in the hydrophobic and hydrophilic interactions characterizing the inactivating bond network, resulting in the motion of the αC-helix through allostery. Some of the clinically identified activating kinase mutations of EGFR also act in a similar fashion, disrupting the inactivating bond network. Our molecular dynamics study reveals a fundamental difference in the sequence of events in EGFR activation in comparison to the Src kinase Hck. This work is funded by NSF grants CBET-0730955 and CBET-0853539.

Protein Folding & Stability II

2302-Pos
Rational Design of Acidic Human Fibroblast Growth Factor (hFGF-1) with Increased Stability and Mitogenic Activity
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Human acidic fibroblast growth factor (hFGF-1) belongs to a superfamily of 22 fibroblast growth factors (FGFs) that have a high affinity to heparin. It is a 16kD protein, which is involved in many cellular functions, such as, cell proliferation, cell differentiation, angiogenesis, tumor growth, and wound healing. In wound healing, the blood coagulation cascade is triggered to clog the wound. One of the proteases that participates in wound healing is thrombin, which converts fibrinogen to fibrin. Thrombin also cleaves wild type hFGF-1 and consequently decreases its wound healing potency. Thrombin is shown to specifically cleave FGF-1 at Arg136. In this context, in the present study we have designed several site-directed mutants at Arg136 to understand the role of this residue not only on the stability of FGF-1 but also on the FGF signaling process. The results of this study will be discussed in detail.

2303-Pos
Design of Novel Affinity Tags to Increase Recovery of Recombinant Proteins in Ther Soluble Forms
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One major challenge in the expression and purification of recombinant proteins is preventing proteins from entering inclusion bodies. While some proteins can be extracted and refolded from the insoluble inclusion bodies others cannot, such as those proteins containing a protein affinity tag. This study will examine those specific challenges and will explore potential solutions to those problems. We aim to exploit properties of osmolytes like betaine, sarcosine, arginine, and proline to design affinity tags that prevent the formation of inclusion bodies during over-expression of proteins in bacterial hosts. Results of the interactions of the designed affinity tag(s) with transition metals, like nickel and cobalt, will be presented. In addition, correlation between recombinant protein expression yields and the length of the affinity tag(s) will also be discussed.

2304-Pos
Direct Observation of Villin Folding in a Microfluidic Mixer
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Villin headpiece subdomain (HP35) is a 35 residues polypeptide that folds on the microsecond timescale. Therefore, it has been an attractive object of protein folding for both computational and experimental studies, with some disagreement between the two methods. In this work, we observed folding of this protein after dilution of high denaturant in an ultrarapid microfluidic mixer far below the thermal midpoint. Tryptophan fluorescence changes were observed at various times beyond mixing from 8 ~1500 μs at temperatures between 10 ~ 40 °C. It is similar with T-jump that fluorescence relaxes can be fit with two exponential decays. Most of the amplitude decays within the mixing time, in agreement with the T-jump observation. But a small population decays on the tens of microsecond timescale. This slow phase is in rough agreement with MD simulations by Ensign et al. (1) and suggests a simple two-state model is not adequate to describe the folding pathway.
1. Ensign DL, PM Kasson, VS Pande. 2007. Heterogeneity even at the speed limit of folding: Large-scale molecular dynamics study of a fast-folding variant of the villin headpiece. Journal of Molecular Biology 374(3):806-816.

2305-Pos
Molecular Basis for the Solvation and Reconstructive Denaturation of Proteins by Detergents
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Detergents are widely used for the biochemical and structural study of proteins. Non-ionic and zwitterionic detergents are used as membrane mimetics, where they solvate the hydrophobic regions of integral membrane proteins. In contrast, ionic detergents such as sodium dodecyl sulphate (SDS) (anionic) and lauryl-trimethylammonium chloride (LTAC) (cationic) are strong protein denaturants that unfold both soluble and membrane proteins. Not only does the SDS-unfolded state have high alpha-helix content, but SDS and other ionic